

VARIANT TYPE II TGF-BETA RECEPTOR FUSION PROTEINS AND METHODS RELATED APPLICATIONS

This is a continuation of PCT PCT/US99/13629, filed June 16, 1999 as a continuation-in-part of U.S.S.N. 60/089,452 filed on 16 June 1998. This entire disclosure of the aforesaid patent applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

The present invention relates to a new soluble TGF-beta receptor fusion proteins and to their uses in treating conditions characterized by an overproduction of TGF- beta, including fibroproliferation

BACKGROUND

Transforming growth factor- beta (TGF- beta) represents a family of polypeptides, of which three are present in mammals, TGF- beta 1, TGF- beta 2 and TGF- beta 3. These factors have global effects on cell growth and differentiation (Roberts and Sporn (1990) Handbk. Exp. Pharm. 95:419-58). There is a growing body of evidence that TGF- beta also modulates the immune process (Wahl et al. (1989) Immunol. Today 10:258-61). In addition to stimulating the congregation of immune cells at the site of injury, TGF- beta also provides strong positive feedback for its own continued synthesis (Kim et al. (1990) Mol.Cell. Biol. 10:1492-1497).

TGF- beta may be a prominent factor in the etiology of fibroproliferative disorders. TGF-beta is known to stimulate cells to produce more proteins, including collagen, biglycan, decorin and fibronectin; and to inhibit enzymes which degrade these proteins. Thus, TGF- beta can cause fibrous tissue to accumulate. For example, in diabetic nephropathy and human mesangial proliferative glomerulonephritis, both fibroproliferative disorders, a prominent and important pathological feature is the accumulation of mesangial matrix (Mauer et al. (1984) J. Clin. Invest, 74: 1143-55). Likewise, postradiation fibrosis is characterized by excess TGF- beta , proliferation of fibroblasts and overproduction of connective tissue (Canney and Dean (1990) Brit. J. Radiol. 63:620-23). Workers in the field have attempted to suppress the effects of TGF- beta by administering anti-TGF-beta antibody. See Border et al. (1990) Nature 346:371-74; Border et al. ((1992) Kidney Int. 41:566-570).

The biological effects of TGF-beta are mediated by several specific cell surface proteins, including the so-called type I, type II, and type III TGF-beta receptors. Suzuki et al. (FEBS Letters 355: 19-22 (1994) and Nikawa (Gene, 149: 367-372 (1994) both discovered a variant of the Type II receptor which in all respects was identical to the Type II receptor except that the variant

had an insertion sequence of 25 amino acids in the predicted ligand-binding domain of the receptor.

Almost all TGF-beta responsive cell lines express both the type I and type II receptors, although the levels of their expression may vary among different cell types. Neither type I nor type II receptor in the absence of the other can mediate a TGF-beta response, although the type II receptor alone is capable of binding TGF-beta.

We previously have developed a fusion protein comprising the Type II TGF-beta receptor and we showed that it decreased fibrosis in a rat blood vessel injury model of restenosis (PCT Publication Number WO 98/48024).

In developing improved TGF-beta inhibitors to treat fibroproliferative disorders, it is important to consider that such inhibitors should have a higher affinity than anti-TGF-beta antibodies. This combination of features would permit much lower doses and increase ease of administration. Moreover, a native protein would not cause cross-species reactions.

SUMMARY OF THE INVENTION

We have discovered that a fusion protein containing a splice variant of TGF-beta type II receptor (previously known but never suspected of having therapeutic utility relative to the non-variant form) is actually more effective than non-variant forms of the fusion protein at blocking TGF beta activity as measured in vitro with the mink lung cell assay, and as measured in vivo by a dosing study. That is, a lower dose of the fusion containing the variant of TGF-beta Type II receptor is as effective as a higher dose of the fusion containing the non-variant form of TGF-beta Type II receptor.

Accordingly, the present invention is directed to novel compositions and methods for the therapeutic treatment of patients having fibroproliferative disorders.

The invention also is directed to recombinant DNA vectors and methods of use thereof. The vectors allow expression of TGF-beta receptor fusion proteins in bacterial and mammalian host cells.

One embodiment of the invention is an isolated variant TGF-beta receptor fusion protein that competitively inhibits binding of TGF-beta to TGF-beta receptor. Preferably, this fusion protein includes the insertion sequence of the splice variant (amino acid residues number 32-57 of SEQ ID NO:2) of TGF-beta Type II receptor linked to a second protein that is not a TGF-beta receptor. It is preferred that the second protein is a constant region of an immunoglobulin.

Isolated polynucleotides are also embodied herein, encoding, on expression, for the variant form of TGF-beta Type II receptor (hereinafter also called "TGF-beta Type IIb receptor") linked to

a second protein that is not a TGF-beta receptor. The preferred polynucleotides are selected from the group consisting of: (a) nucleotides 832 to 2070 of SEQ ID NO.: 1, (b) polynucleotides that hybridize to these sequences under standard hybridization conditions and that encode a protein having the TGF-beta inhibitory activity of a TGF-beta Type IIb receptor fusion protein; and (c)
5 polynucleotides that code on expression for a protein encoded by any of the foregoing polynucleotide sequences.

Pharmaceutical compositions of the invention are exemplified by a variant TGF-beta receptor fusion protein in a pharmaceutically acceptable carrier, the fusion protein in an amount sufficient to competitively inhibit binding of TGF-beta to a TGF-beta ligand.

10 Methods of the invention include a method for producing a variant TGF-beta receptor fusion protein, comprising culturing a host cell containing a vector of the invention, allowing said cell to express the fusion protein, isolating and purifying the fusion protein.

One embodiment is a method for lowering the levels of TGF-beta in an individual which comprises administering to the individual an effective amount of a TGF-beta antagonist that is a
15 variant TGF-beta receptor fusion protein comprising the sequence of amino acids of SEQ ID NO: 2.

Another embodiment is a method for lowering the levels of TGF-beta in an individual having arthritis, which comprises administering to the individual an effective amount of a TGF-beta antagonist that a variant TGF-beta receptor fusion protein comprising the sequence of amino
20 acids of SEQ ID NO: 2.

In one embodiment, the invention provides a method for treating an individual for a medical condition associated with a fibroproliferative disorder. The method provides for the parenteral, oral or local administration of a sufficient amount of soluble variant form of TGF-beta receptor fusion protein to the individual to reduce or ameliorate the disorder and/or block activity
25 of TGF- beta activity in the individual. In yet another embodiment, the method of the present invention provides for administration of the variant form of TGF- beta receptor fusion protein by intravenous, intraocular, intraarticular, transdermal and enteral methods. In another embodiment of the present invention, the variant TGF- beta receptor fusion protein is administered to patients with collagen vascular disorders, such as systemic sclerosis, polymyositis, scleroderma,
30 dermatomyositis, or systemic lupus erythematosus.

In yet another embodiment of the present invention, the variant form of TGF- beta receptor fusion protein is administered to subjects with with intraocular and pulmonary fibrosis, in particular to those subjects with fibrotic conditions derived from restenosis. In a further

embodiment, the TGF- beta receptor fusion protein is administered to patients with diabetic nephropathy, glomerulonephritis, proliferative vitreoretinopathy, and rheumatoid arthritis.

In still another embodiment, the method of the present invention provides for treating a wound in an individual to avoid overproduction of connective tissue formation which is

5 associated with upregulation of TGF- beta. The method provides for administration of a sufficient amount of the variant TGF- beta receptor fusion protein to the individual to reduce the amount or activity of TGF- beta in the individual. In further embodiments, the types of wounds include surgical incisions, trauma-induced lacerations and wounds involving the peritoneum for which the overproduction of connective tissue formation is abdominal adhesions. In a further
10 embodiment, the overproduction of connective tissue formations which are avoided include scars, including those where the scar involves restenosis of blood vessels, and hypertrophic scars, and keloids.

In another embodiment, the invention provides a method of preventing postradiation fibrosis in an individual undergoing or about to undergo radiation therapy. TGF- beta receptor
15 fusion protein of the invention is administered in an amount sufficient to prevent overproduction of fibrous tissue formation.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. The accompanying drawings are included to provide a
20 further understanding of the invention and are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention, and together with the description serve to explain the principles of the invention.

DETAILED DESCRIPTION

All references cited in the Detailed Description are incorporated herein by references, unless
25 stipulated otherwise. The following terms are used herein:

I. Definitions

"Recombinant," as used herein, means that a protein is derived from recombinant (e.g., microbial or mammalian) expression systems. "Microbial" refers to recombinant proteins made in bacterial or fungal (e.g., yeast) expression systems. As a product, "recombinant microbial"
30 defines a protein produced in a microbial expression system which is essentially free of native endogenous substances. Protein expressed in most bacterial cultures, e.g., E. coli, will be free of glycan. Protein expressed in yeast may have a glycosylation pattern different from that expressed in mammalian cells.

"Biologically active," as used throughout the specification as a characteristic of TGF-beta receptors, means that a particular molecule shares sufficient amino acid sequence homology with the embodiments of the present invention disclosed herein to be capable of binding detectable quantities of TGF-beta and/or transmitting a TGF-beta stimulus to a cell, for example, as a component of a hybrid receptor construct, or cross-reacting with anti-TGF-beta receptor antibodies raised against TGF-beta receptor from natural (i.e., nonrecombinant) sources. Preferably, biologically active TGF-beta receptor fusions within the scope of the present invention are capable of binding greater than 0.1 nmoles TGF-beta per nanomole (nmole) receptor, and most preferably, greater than 0.5 nmole TGF-beta per nmole receptor in standard binding assays (see below).

Similarly, a "TGF-beta binding molecule", as used herein, is any molecule exhibiting the same, or substantially the same, biological activity as a TGF-beta receptor fusion protein.

"Individual" means a living organism, including humans, other mammals and any other animals which produce TGF- beta .

"TGF- beta overproduction" as used herein is an amount of TGF- beta present in serum or tissue which is significantly above the normal level. More preferably, TGF-beta overproduction is a level between about 2 and about 20 times normal. Even more preferably, TGF- beta overproduction is a level between about 2 and about 15 times normal. For example, Deguchi measured 24-hour TGF- beta production of bronchoalveolar cells and reported normal levels of 410 +/- 225 pg/10⁷ cells against excess TGF- beta production of 1288 +/- 453 pg/10⁷ cells in systemic lupus erythematosus and 1417 +/- 471 pg/10⁷ cells in scleroderma ((1992) Ann. Rheum. Dis. 51:362-65). TGF- beta overproduction can be determined, in combination with normal levels, by measurement of the TGF- beta protein, of TGF- beta MRNA, or of products whose synthesis is stimulated by TGF- beta , such as collagen.

"Connective tissue" is fibrous tissue characterized by the presence of fibroblasts and fibrous proteins such as collagen and elastin.

A "fibroproliferative disorder" is characterized by proliferation of fibroblasts plus the corresponding overexpression of extracellular matrix such as fibronectin, laminin and collagen.

A "therapeutic composition" as used herein is defined as comprising the proteins of the invention and other physiologically compatible ingredients. The therapeutic composition may contain excipients such as water, minerals and carriers such as protein.

"A sufficient amount" of the proteins of the invention as used herein refers to the amount of TGF- beta receptor fusion protein that neutralizes the biologic activity of excess TGF- beta . It

may be determined by (1) suitable clinical variables of improvement, (2) pathologic evaluation of the effects on fibrosis and/or immunosuppression or prevention of fibrosis, or (3) a direct inhibition of TGF- beta.

“amino acid”- a monomeric unit of a peptide, polypeptide, or protein. There are
5 twenty amino acids found in naturally occurring peptides, polypeptides and proteins, all of which are L-isomers. The term also includes analogs of the amino acids and D-isomers of the protein amino acids and their analogs.

“protein”- any polymer consisting essentially of any of the 20 amino acids. Although
10 “polypeptide” is often used in reference to relatively large polypeptides, and “peptide” is often used in reference to small polypeptides, usage of these terms in the art overlaps and is varied. The term “protein” as used herein refers to peptides, proteins and polypeptides, unless otherwise noted.

“functional equivalent” of an amino acid residue is an amino acid having similar
15 physico-chemical properties as the amino acid residue that was replaced by the functional equivalent.

“fusion”- refers to a co-linear, covalent linkage of two or more proteins or fragments thereof via their individual peptide backbones, most preferably through genetic expression of a polynucleotide molecule encoding those proteins.

“mutant” - any change in the genetic material of an organism, in particular any
20 change (i.e., deletion, substitution, addition, or alteration) in a wild-type polynucleotide sequence or any change in a wild-type protein.

“wild-type” - the naturally-occurring polynucleotide sequence of an exon of a protein, or a portion thereof, or protein sequence, or portion thereof, respectively, as it normally exists *in vivo*.

25 “standard hybridization conditions”- salt and temperature conditions substantially equivalent to 0.5 X SSC to about 5 X SSC and 65 ° C for both hybridization and wash. The term “standard hybridization conditions” as used herein is therefore an operational definition and encompasses a range of hybridization conditions. Higher stringency conditions may, for example, include hybridizing with plaque screen buffer (0.2% polyvinylpyrrolidone, 0.2% Ficoll
30 400; 0.2% bovine serum albumin, 50 mM Tris-HCl (pH 7.5); 1 M NaCl; 0.1% sodium pyrophosphate; 1 % SDS); 10% dextran sulphate, and 100 µg/ml denatured, sonicated salmon sperm DNA at 65 ° C for 12-20 hours, and washing with 75 mM NaCl/7.5 mM sodium citrate (0.5 x SSC)/1% SDS at 65° C. Lower stringency conditions may, for example, include

hybridizing with plaque screen buffer, 10% dextran sulphate and 110 µg/ml denatured, sonicated salmon sperm DNA at 55 ° C for 12-20 hours, and washing with 300 mM NaCl/30mM sodium citrate (2.0 X SSC)/1% SDS at 55 ° C. See also **Current Protocols in Molecular Biology**, John Wiley & Sons, Inc. New York, Sections 6.3.1-6.3.6, (1989).

5 "expression control sequence"- a sequence of polynucleotides that controls and regulates expression of genes when operatively linked to those genes.

"operatively linked"- a polynucleotide sequence (DNA, RNA) is operatively linked to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that polynucleotide sequence. The term "operatively linked"
10 includes having an appropriate start signal (e.g., ATG) in front of the polynucleotide sequence to be expressed and maintaining the correct reading frame to permit expression of the polynucleotide sequence under the control of the expression control sequence and production of the desired polypeptide encoded by the polynucleotide sequence.

"expression vector"- a polynucleotide, such as a DNA plasmid or phage (among other
15 common examples) which allows expression of at least one gene when the expression vector is introduced into a host cell. The vector may, or may not, be able to replicate in a cell.

"Isolated" (used interchangeably with "substantially pure")- when applied to nucleic acid i.e., polynucleotide sequences, that encode polypeptides, means an RNA or DNA polynucleotide, portion of genomic polynucleotide, cDNA or synthetic polynucleotide which, by
20 virtue of its origin or manipulation: (i) is not associated with all of a polynucleotide with which it is associated in nature (e.g., is present in a host cell as an expression vector, or a portion thereof); or (ii) is linked to a nucleic acid or other chemical moiety other than that to which it is linked in nature; or (iii) does not occur in nature. By "isolated" it is further meant a polynucleotide sequence that is: (i) amplified *in vitro* by, for example, polymerase chain reaction (PCR); (ii)
25 chemically synthesized; (iii) recombinantly produced by cloning; or (iv) purified, as by cleavage and gel separation.

Thus, "substantially pure nucleic acid" is a nucleic acid which is not immediately contiguous with one or both of the coding sequences with which it is normally contiguous in the naturally occurring genome of the organism from which the nucleic acid is derived. Substantially
30 pure DNA also includes a recombinant DNA which is part of a hybrid gene encoding additional sequences.

"Isolated" (used interchangeably with "substantially pure")- when applied to polypeptides means a polypeptide or a portion thereof which, by virtue of its origin or

manipulation: (i) is present in a host cell as the expression product of a portion of an expression vector; or (ii) is linked to a protein or other chemical moiety other than that to which it is linked in nature; or (iii) does not occur in nature. By "isolated" it is further meant a protein that is : (i) chemically synthesized; or (ii) expressed in a host cell and purified away from associated proteins. The term generally means a polypeptide that has been separated from other proteins and nucleic acids with which it naturally occurs. Preferably, the polypeptide is also separated from substances such as antibodies or gel matrices (polyacrylamide) which are used to purify it.

"heterologous promoter"- as used herein is a promoter which is not naturally associated with a gene or a purified nucleic acid.

"Homologous"- as used herein is synonymous with the term "identity" and refers to the sequence similarity between two polypeptides, molecules or between two nucleic acids. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit (for instance, if a position in each of the two DNA molecules is occupied by adenine, or a position in each of two polypeptides is occupied by a lysine), then the respective molecules are homologous at that position. The percentage homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared x 100. For instance, if 6 of 10 of the positions in two sequences are matched or are homologous, then the two sequences are 60% homologous. By way of example, the DNA sequences CTGACT and CAGGTT share 50% homology (3 of the 6 total positions are matched). Generally, a comparison is made when two sequences are aligned to give maximum homology. Such alignment can be provided using, for instance, the method of Needleman et al., *J. Mol Biol.* 48: 443-453 (1970), implemented conveniently by computer programs such as the Align program (DNASTar, Inc.). Homologous sequences share identical or similar amino acid residues, where similar residues are conservative substitutions for, or "allowed point mutations" of, corresponding amino acid residues in an aligned reference sequence. In this regard, a "conservative substitution" of a residue in a reference sequence are those substitutions that are physically or functionally similar to the corresponding reference residues, e.g., that have a similar size, shape, electric charge, chemical properties, including the ability to form covalent or hydrogen bonds, or the like. Particularly preferred conservative substitutions are those fulfilling the criteria defined for an "accepted point mutation" in Dayhoff et al., 5: **Atlas of Protein Sequence and Structure**, 5: Suppl. 3, chapter 22: 354-352, Nat. Biomed. Res. Foundation, Washington, D.C. (1978).

The terms "peptide(s)", "protein(s)" and "polypeptide(s)" are used interchangeably herein. The terms "polynucleotide sequence" and "nucleotide sequence" are also used interchangeably herein.

Practice of the present invention will employ, unless indicated otherwise, conventional techniques of cell biology, cell culture, molecular biology, microbiology, recombinant DNA, protein chemistry, and immunology, which are within the skill of the art. Such techniques are described in the literature. See, for example, **Molecular Cloning: A Laboratory Manual**, 2nd edition. (Sambrook, Fritsch and Maniatis, eds.), Cold Spring Harbor Laboratory Press, 1989; **DNA Cloning**, Volumes I and II (D.N. Glover, ed), 1985; **Oligonucleotide Synthesis**, (M.J. Gait, ed.), 1984; U.S. Patent No. 4,683,195 (Mullis et al.); **Nucleic Acid Hybridization** (B.D. Hames and S.J. Higgins, eds.), 1984; **Transcription and Translation** (B.D. Hames and S.J. Higgins, eds.), 1984; **Culture of Animal Cells** (R.I. Freshney, ed). Alan R. Liss, Inc., 1987; **Immobilized Cells and Enzymes**, IRL Press, 1986; **A Practical Guide to Molecular Cloning** (B. Perbal), 1984; **Methods in Enzymology**, Volumes 154 and 155 (Wu et al., eds), Academic Press, New York; **Gene Transfer Vectors for Mammalian Cells** (J.H. Miller and M.P. Calos, eds.), 1987, Cold Spring Harbor Laboratory; **Immunochemical Methods in Cell and Molecular Biology** (Mayer and Walker, eds.), Academic Press, London, 1987; **Handbook of Experimental Immunology**, Volumes I-IV (D.M. Weir and C.C. Blackwell, eds.), 1986; **Manipulating the Mouse Embryo**, Cold Spring Harbor Laboratory Press, 1986.

II. General Properties of Isolated Proteins and Polynucleotides as TGF-beta Antagonists

A. Soluble TGF-beta receptor variants as fusions and their Analogs

The present invention utilizes isolated and purified splice variants of TGF-beta antagonist polypeptides. The isolated and purified TGF-beta antagonist polypeptides used in this invention are substantially free of other contaminating materials of natural or endogenous origin and contain less than about 1% by mass of protein contaminants residual of production processes. The TGF-beta antagonist polypeptides used in this invention are optionally without associated native-pattern glycosylation.

As used herein, the term "variant TGF-beta receptor" refers to proteins having amino acid sequences which are substantially similar to the splice variant TGF-beta receptor or TGF-beta binding molecule sequences as shown in SEQ ID NO 2 and which are capable of binding TGF-beta and inhibiting TGF-beta from binding to cell membranes containing TGF-beta receptor. The preferred TGF-beta receptor is mature full-length TGF-beta receptor Type IIb splice variant

whose sequence was first identified by Suzuki et al., FEBS Letters, 335: 19-22 (1994: See their Figure 1). Generally, TGF- beta receptor Type IIb is a membrane-bound protein with an intracellular domain, transmembrane domain and extracellular portion which binds to TGF- beta. Human TGF- beta receptor Type II has been determined to have the amino acid sequence shown in Lin et al., Cell: 68, 775-785 (1992), and corrected as shown in U.S. Patent 5,693,607.

The preferred TGF-beta receptors of the present invention are soluble forms of TGF-beta receptor Type IIb, as well as soluble TGF-beta Type IIb binding molecules. Soluble TGF-beta receptors include, for example, analogs or subunits of native proteins having at least 20 amino acids and which exhibit at least some biological activity in common with TGF-beta receptor Type IIb or TGF-beta binding molecules.

The terms "TGF-beta Type IIb receptor fusion protein" or "TGF-beta RIIb:Fc" or "TGF- δ RIIB:Fc" are used interchangeably and represent the most preferred embodiments of the compositions of the present invention. This fusion protein contains at least part of the TGF-beta receptor (Type IIb) splice variant linked to at least part of a second protein that is not a TGF-beta receptor. The second protein may be the constant region of an immunoglobulin (preferably IgG, most preferably IgG1) or may be a portion thereof such as the hinge, C_H2 or C_H3. The preferred fusions of the invention are homodimers, connected to each other by disulfide linkages but heterodimeric forms are also means to be within the scope of the invention. See Section IV.

Thus, the most preferred compositions of the present invention are TGF-beta antagonists that are soluble fusion proteins such as recombinant chimeric antibody molecules having TGF-beta receptor sequences substituted for the variable domains of either or both of the immunoglobulin molecule heavy and light chains and having unmodified constant region domains. For example, chimeric TGF-betaRIIb/IgG1 is produced from chimeric genes: a TGF-beta RIIb/human gamma 1 heavy chain chimera. Following transcription and translation of the chimeric gene, the gene product assembles into a homodimer having TGF-beta receptor displayed bivalently. Such polyvalent forms of TGF-beta receptor may have enhanced binding affinity for TGF-beta ligand.

The soluble TGF-beta receptor constructs of the invention are devoid of a transmembrane region (and are secreted from the cell) but retain the ability to bind TGF-beta. Various bioequivalent protein and amino acid analogs have an amino acid sequence corresponding to all or part of the extracellular region of the Type IIb TGF-beta receptor such as the polypeptide sequence encoded by nucleotides 832 to 2070 of SEQ ID NO: 1 or those comprising amino acid residues 1 to 185 of SEQ ID NO: 2 or amino acid sequences substantially similar or are at least

60% homologous to these same amino acid sequences of SEQ ID NO: 2 and which are biologically active in that they bind to TGF-beta ligand. Equivalent TGF-beta IIb receptors include polypeptides which vary from these sequences by one or more substitutions, deletions, or additions, and which retain the ability to bind TGF-beta or inhibit TGF-beta signal transduction activity via cell surface bound TGF-beta receptor proteins. Inhibition of TGF-beta signal transduction activity can be determined by transfecting cells with recombinant TGF-beta IIb receptor DNAs to obtain recombinant receptor expression. The cells are then contacted with TGF-beta and the resulting metabolic effects examined. If an effect results which is attributable to the action of the ligand, then the recombinant receptor has signal transduction activity. Exemplary procedures for determining whether a polypeptide has signal transduction activity are disclosed by Idzerda et al., J. Exp. Med. 171:861 (1990); Curtis et al., Proc. Natl. Acad. Sci.U.S.A. 86:3045 (1989); Prywes et al., EMBO J. 5:2179 (1986) and Chou et al., J. Biol. Chem. 262:1842 (1987). Alternatively, primary cells or cell lines which express an endogenous TGF-beta receptor and have a detectable biological response to TGF-beta could also be utilized.

A "fragment" of the proteins of this invention is a portion or all of a protein which is capable of binding TGF- beta. Preferably, this fragment has a high affinity for TGF- beta. Preferred fragments of fusion proteins are protein fragments comprising at least part of the extracellular portion of the TGF- beta receptor Type IIb bound to at least part of a constant region of an immunoglobulin . Preferably, the affinity is in the range of about 10^{-11} to 10^{-12} M, although the affinity may vary considerably with fragments of different sizes, ranging from 10^{-7} to 10^{-13} M. In one embodiment, the fragment is about 10-110 amino acids in length and comprises the TGF-beta binding site linked to at least part of a constant region of an immunoglobulin.

If the amino acid sequence of the extracellular domain of the splice variant form of TGF-beta receptor (e.g. amino acids 1 to 185 of SEQ ID NO: 2 or that polypeptide encoded by nucleotides 832 to 2070 of SEQ ID NO: 1) is used in the fusion proteins, the amino acid sequence resembles that of the entire extracellular portion of the Type IIb receptor. When smaller TGF beta - receptor Type IIb portions are employed, they resemble various portions of the extracellular portions of the Type IIb TGF- beta receptor, so long as they bind TGF- beta with high affinity. Although the sequence of a given "fragment" is based most preferably on the variant TGF- beta receptor IIb extracellular region, the definition also comprises analogs of proteins of the invention which have high affinity for TGF- beta. Such analogs include those made by conservative substitutions of amino acids, as well as those made by mutated cells synthesizing the fragment.

The polypeptides include an amino acid sequence at least 60%, 80%, 90%, 95%, 98%, or 99% homologous to an amino acid sequence from SEQ ID NO: 2. The polypeptide can also include an amino acid sequence essentially the same as an amino acid sequence in SEQ ID NO: 2. The polypeptide is at least 5, 10, 20, 50, 100, or 150 amino acids in length and includes at least 5,
 5 preferably at least 10, more preferably at least 20, most preferably at least 50, 100, or 150 contiguous amino acids from SEQ ID NO: 2.

The polypeptide can be made entirely by synthetic means or can be expressed in systems, e.g., cultured cells, which result in substantially the same posttranslational modifications present when the protein is expressed in a native cell, or in systems which result in the omission of
 10 posttranslational modifications present when expressed in a native cell.

B. Isolated Polynucleotide Sequences

Preferred polynucleotides of the invention are derived from polynucleotides that encode, upon expression, the splice variant Type IIb TGF-beta receptor (e.g. polynucleotides 832 to 2070
 15 of SEQ ID NO.: 1) See also Suzuki et al, supra. A cDNA encoding a TGF-beta receptor Type IIb molecule ID NO.: 1) is a complementary DNA (cDNA) sequence encoding for the splice variant TGF-beta Type II receptor. Moreover, the isolated, polynucleotides, for example the expression vector depicted in SEQ ID NO. 1 can be altered to provide for functionally equivalent polynucleotides. A polynucleotide is "functionally equivalent" compared with polynucleotides 832
 20 to 2070 of SEQ ID NO: 1 (i.e., encoding amino acids 1 to 185 of SEQ ID NO: 2) or polynucleotides such as SEQ ID NO.: 1 if it satisfies at least one of the following conditions: (a): the "functional equivalent" is a polynucleotide that hybridizes to at least a portion of SEQ ID NO. 1 or at least a portion of the polynucleotide encoding amino acids 1 to 185 of SEQ ID NO: 2 under standard hybridization conditions. Most preferably, it encodes a TGF-beta receptor protein having
 25 the same biological activity as Type IIb TGF-beta receptor; and (b) the "functional equivalent" is a polynucleotide that codes on expression for an amino acid sequence encoded by at least amino acids 1 to 185 of SEQ ID NO. 2.

Due to the degeneracy of the nucleotide coding sequences, other polynucleotides may be used to encode variant TGF-beta receptors or fusions thereof. For TGF-beta Type IIb receptor,
 30 these include all, or portions of DNA encoding SEQ ID NO.: 1, which are altered by the substitution of different codons that encode the same amino acid residue within the sequence, thus producing a silent change. Such altered sequences are regarded as equivalents of SEQ ID NO.: 1. For example, Phe(f) is coded for by two codons, TTC or TTT, Try (Y) is coded for by TAC or

TAT and His (H) is coded for by CAC or CAT. On the other hand, Trp (W) is coded for by a single codon, TGG. Accordingly, it will be appreciated that for a given DNA sequence encoding a particular protein there will be many DNA degenerate sequences that will code for it. These degenerate DNA sequences are considered within the scope of this invention.

5 Fusion proteins of the invention incorporating the human TGF-beta Type IIb receptor are shown in SEQ ID NOS: 1 and 2 for the cDNA and deduced amino acids sequences, respectively.

The preferred TGF-beta RIIb:Fc proteins of the invention include the novel "junction" DNA sequence SEQ ID NO: 5 and amino acid SEQ ID NO: 6. SEQ ID NO: 5 represents the 11 triplet codons on either side of the junction between the TGF-betaRIIb DNA and the DNA of the immunoglobulin. In SEQ ID NO: 5, the corresponding junction begins at the triplet encoding amino acid 185 of SEQ ID NO 2 and ends at the triplet encoding amino acid 186 of SEQ ID NO: 2. The corresponding deduced amino acid "junction" sequence is represented in SEQ ID NO: 6 in which the end of the TGF-beta receptor sequence is the leucine (amino acid 185) and the beginning of the immunoglobulin sequence is the valine immediately thereafter.

15 It might be that SEQ ID NO: 5 is the minimum DNA needed for hybridization under standard conditions to any DNA sequence encoding any TGF-beta receptorIIb:Fc fusion protein. Nevertheless, provided that the whole probe hybridizes to both sides of the junction and both sides of the TGF-beta receptor/constant region junction participate in the hybridization, smaller sequences may exist. Furthermore, persons having ordinary skill in the art will understand that DNA sequences larger than SEQ ID NO: 5 will be suitable for hybridization as well. One of ordinary skill in the art can test if a particular probe such as SEQ ID NO: 5 are capable of hybridizing on both sides of the junction by labelling the 5' end of either a single strand sense oligonucleotide or a single strand anti-sense oligonucleotide with an appropriately labelled phosphate of ATP using polynucleotide kinase. A sequence of the invention must hybridize to, and thus be labelled by both oligonucleotide probes. It is further understood that the invention encompasses fully degenerate sequences encoding the junction SEQ ID NO: 5.

The vector containing the TGF-beta RIIb:Fc cDNA clone, was used to express and purify soluble TGF-beta RIIb:Fc.

30 Other mammalian variant TGF-beta receptor cDNAs may be isolated by using an appropriate human TGF-beta IIb receptor DNA sequence as a probe for screening a particular mammalian cDNA library by cross-species hybridization. Mammalian TGF-beta receptor used in the present invention includes, by way of example, primate, human, murine, canine, feline, bovine, equine and porcine TGF-beta IIb receptor. Mammalian TGF-beta IIb receptors can be obtained by

cross species hybridization, using a single stranded cDNA derived from the human TGF-beta receptor DNA sequence as a hybridization probe to isolate TGF-beta IIb receptor cDNAs from mammalian cDNA libraries.

5 **III. Production of Recombinant Variant Polypeptides**

The isolated polypeptides described herein can be produced by any suitable method known in the art. Such methods range from direct protein synthetic methods to constructing a DNA sequence encoding isolated polypeptide sequences and expressing those sequences in a suitable transformed host. For example, cDNA may be obtained by screening a human cDNA library
10 with the appropriate labeled DNA fragment encoding at least a portion of the polypeptide of SEQ ID NO: 2 and identifying positive clones by autoradiography. Further rounds of plaque purification and hybridization are performed using conventional methods.

In one embodiment of a recombinant method, a DNA sequence is constructed by isolating or synthesizing a DNA sequence encoding a variant TGF-beta IIb protein of interest. Optionally, the
15 sequence may be mutagenized by site-specific mutagenesis to provide functional analogs thereof. See, e.g. Zoeller et al., Proc.-Nat Acad. Sci. USA 81:5662-5066 (1984) and United States Patent 4,588,585. Another method of constructing a DNA sequence encoding a polypeptide of interest would be by chemical synthesis using an oligonucleotide synthesizer. Such oligonucleotides may be preferably designed based on the amino acid sequence of the desired polypeptide, and
20 preferably selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced.

Standard methods may be applied to synthesize an isolated polynucleotide sequence encoding a isolated polypeptide of interest. For example, a complete amino acid sequence may be used to construct a back-translated gene. See Maniatis et al., *supra*. Further, a DNA oligomer containing
25 a nucleotide sequence coding for the particular isolated polypeptide may be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide may be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

Once assembled (by synthesis, site-directed mutagenesis or another method), the mutant
30 DNA sequences encoding a particular isolated polypeptide of interest will be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly may be confirmed by nucleotide sequencing, restriction mapping, and expression of a biologically active polypeptide in a suitable

host. As is well known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

5 A. Expression of Recombinant TGF-beta IIb Receptor

Recombinant expression vectors are preferably used to amplify or express DNA encoding variant TGF-beta IIb receptor fusions. Recombinant expression vectors are replicable DNA constructs which have synthetic or cDNA-derived DNA fragments encoding TGF-beta IIb receptor fusions or bioequivalent analogs operatively linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences, as described in detail below. Such regulatory elements may include an operator sequence to control transcription. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated. DNA regions are operatively linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operatively linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. Generally, operatively linked means contiguous and, in the case of secretory leaders, contiguous and in reading frame. Structural elements intended for use in yeast expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

DNA sequences encoding mammalian TGF-beta IIb receptors which are to be expressed as a fusion in microorganisms may preferably contain introns that could prematurely terminate transcription of DNA into mRNA since such premature termination of transcription may be desirable, for example, where it would result in mutants having advantageous C-terminal truncations, for example, deletion of a transmembrane region to yield a soluble receptor not

bound to the cell membrane. Due to code degeneracy, there can be considerable variation in nucleotide sequences encoding the same amino acid sequence. As discussed above, other embodiments include sequences capable of hybridizing to the sequences of the provided cDNA under standard conditions (50 degrees C., 2 x SSC) and other sequences hybridizing or
 5 degenerate to those which encode biologically active proteins of the invention.

The choice of expression control sequence and expression vector will depend upon the choice of host. A wide variety of expression host/vector combinations may be employed. Useful expression vectors for eukaryotic hosts, include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and
 10 cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *Escherichia. coli*, including pCR1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as M13 and filamentous single-stranded DNA phages. Preferred *E. coli* vectors include pL vectors containing the lambda phage pL promoter (U.S. Patent 4,874,702), pET vectors containing the T7 polymerase promoter (Studier et al., **Methods in**
 15 **Enzymology** 185: 60-89, 1990 1) and the pSP72 vector (Kaelin et al., *supra*). Useful expression vectors for yeast cells, for example, include the 2 T and centromere plasmids.

In addition, any of a wide variety of expression control sequences may be used in these vectors. Such useful expression control sequences include the expression control sequences associated with structural genes of the foregoing expression vectors. Examples of useful
 20 expression control sequences include, for example, the early and late promoters of SV40 or adenovirus, the *lac* system, the *trp* system, the *TAC* or *TRC* system, the major operator and promoter regions of phage lambda, for example pL, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast *alpha*-mating system and other sequences
 25 known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses, and various combinations thereof.

Recombinant TGF-beta IIb receptor DNA or recombinant TGF-beta RIIb:Fc DNA is expressed or amplified in a recombinant expression system comprising a substantially homogeneous monoculture of a suitable host which have stably integrated (by transformation or
 30 transfection) a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit as a component of a resident plasmid. Generally, cells constituting the system are the progeny of a single ancestral transformant. Recombinant expression systems as defined

herein will express heterologous protein upon induction of the regulatory elements linked to the DNA sequence or synthetic gene to be expressed.

Suitable host cells for expression of mammalian TGF-beta IIb receptor and its fusion proteins include prokaryotes, yeast or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or bacilli. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed to produce mammalian TGF-beta IIb receptor and TGF-beta RIIB:Fc using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., 1985).

Recombinant proteins of the invention may also be expressed in yeast hosts, preferably from the *Saccharomyces* species, such as *S. cerevisiae*. Yeast of other genera, such as *Pichia* or *Kluyveromyces* may also be employed. Yeast vectors will generally contain an origin of replication from the 2 mu yeast plasmid or an autonomously replicating sequence (ARS), promoter, DNA encoding proteins of the invention and DNA sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of replication and selectable marker permitting transformation of both yeast and *E. coli*, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 or URA3 gene, which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the TRP1 or URA3 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan or uracil.

Suitable promoter sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., J. Biol. Chem. 255:2073, 1980) or other glycolytic enzymes (Hess et al., J. Adv. Enzyme Reg. 7:149, 1968; and Holland et al., Biochem. 7:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPA 73,657. Preferred yeast vectors can be assembled using DNA sequences from pUC18 for selection and replication in *E. coli*. (Amp^r gene and origin of replication) and yeast DNA

sequences including a glucose-repressible ADH2 promoter and *alpha*-factor secretion leader. The ADH2 promoter has been described by Russell et al. (J. Biol. Chem. 258:2674, 1982) and Beier et al. (Nature 300:724, 1982). The yeast *alpha*-factor leader, which directs secretion of heterologous proteins, can be inserted between the promoter and the structural gene to be expressed. See, e.g., Kurjan et al., Cell 30:933, 1982; and Bitter et al., Proc. Natl. Acad. Sci. U.S.A. 81:5330, 1984. The leader sequence may be modified to contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes.

Suitable yeast transformation protocols are known to those of skill in the art; an exemplary technique is described by Hinnen et al., Proc. Natl. Acad. Sci. U.S.A. 75:1929, 1978 as described by Sherman et al., Laboratory Course Manual for Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1986.

Various mammalian or insect cell culture systems are also advantageously employed to express recombinant protein. Expression of recombinant proteins in mammalian cells is particularly preferred because such proteins are generally correctly folded, appropriately modified and completely functional. Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (Cell 23:175, 1981), and other cell lines capable of expressing an appropriate vector including, for example, L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors may comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, Bio/Technology 6:47 (1988).

Recombinant expression vectors comprising cDNAs are stably integrated into a host cell's DNA. Elevated levels of expression product is achieved by selecting for cell lines having amplified numbers of vector DNA. Cell lines having amplified numbers of vector DNA are selected, for example, by transforming a host cell with a vector comprising a DNA sequence which encodes an enzyme which is inhibited by a known drug. The vector may also comprise a DNA sequence which encodes a desired protein. Alternatively, the host cell may be co-transformed with a second vector which comprises the DNA sequence which encodes the desired protein. The transformed or co-transformed host cells are then cultured in increasing concentrations of the known drug, thereby selecting for drug-resistant cells. Such drug-resistant

cells survive in increased concentrations of the toxic drug by over-production of the enzyme which is inhibited by the drug, frequently as a result of amplification of the gene encoding the enzyme. Where drug resistance is caused by an increase in the copy number of the vector DNA encoding the inhibitable enzyme, there is a concomitant co-amplification of the vector DNA
5 encoding the desired protein in the host cell's DNA.

A preferred system for such co-amplification uses the gene for dihydrofolate reductase (DHFR), which can be inhibited by the drug methotrexate (MTX). To achieve co-amplification, a host cell which lacks an active gene encoding DHFR is either transformed with a vector which comprises DNA sequence encoding DHFR and a desired protein, or is co-transformed with a
10 vector comprising a DNA sequence encoding DHFR and a vector comprising a DNA sequence encoding the desired protein. The transformed or co-transformed host cells are cultured in media containing increasing levels of MTX, and those cells lines which survive are selected.

A particularly preferred co-amplification system uses the gene for glutamine synthetase (GS), which is responsible for the synthesis of glutamate and ammonia using the hydrolysis of
15 ATP to ADP and phosphate to drive the reaction. GS is subject to inhibition by a variety of inhibitors, for example methionine sulfoximine (MSX). Thus, proteins of the invention can be expressed in high concentrations by co-amplifying cells transformed with a vector comprising the DNA sequence for GS and a desired protein, or co-transformed with a vector comprising a DNA sequence encoding GS and a vector comprising a DNA sequence encoding the desired protein,
20 culturing the host cells in media containing increasing levels of MSX and selecting for surviving cells. The GS co-amplification system, appropriate recombinant expression vectors and cells lines, are described in the following PCT applications: WO 87/04462, WO 89/01036, WO 89/10404 and WO 86/05807.

Recombinant proteins are preferably expressed by co-amplification of DHFR or GS in a
25 mammalian host cell, such as Chinese Hamster Ovary (CHO) cells, or alternatively in a murine myeloma cell line, such as SP2/0-Ag14 or NS0 or a rat myeloma cell line, such as YB2/3.0-Ag20, disclosed in PCT applications WO/89/10404 and WO 86/05807.

It should be understood that not all vectors and expression control sequences will function equally well to express a given isolated polypeptide. Neither will all hosts function equally well
30 with the same expression system. However, one of skill in the art may make a selection among these vectors, expression control systems and hosts without undue experimentation.

B. Purification of Recombinant TGF-betaRIIb:Fc

The proteins produced by a transformed host can be purified according to any suitable method. Such standard methods include chromatography (e.g., ion exchange, affinity and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexahistidine, maltose binding domain, influenza coat sequence and glutathione-S-transferase can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Isolated proteins can also be physically characterized using such techniques as proteolysis, nuclear magnetic resonance and x-ray crystallography.

For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise a TGF-beta or lectin or antibody molecule or Protein A bound to a suitable support. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a TGF-betaRIIb:Fc composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant mammalian TGF-beta receptor can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Fermentation of yeast which express mammalian variant TGF-beta IIb receptor fusion as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be purified by methods analogous to those disclosed by Urdal et al.

(J. Chromatog. 296:171, 1984). This reference describes two sequential, reversed-phase HPLC steps for purification of recombinant human GM-CSF on a preparative HPLC column.

C. Production of Fragments and Analogs

Fragments of an isolated protein (e.g., fragments of SEQ ID NO: 8) can also be produced efficiently by recombinant methods, by proteolytic digestion, or by chemical synthesis, using methods known to those of skill in the art. In recombinant methods, internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end (for a terminal fragment) or both ends (for an internal fragment) of a DNA sequence which encodes for the isolated polypeptide. Expression of the mutagenized DNA produces polypeptide fragments. Digestion with "end nibbling" endonucleases can also generate DNAs which encode an array of fragments. DNAs which encode fragments of a protein can also be generated by random shearing, restriction digestion or a combination or both.

TGF-beta IIb receptor fragments can be generated directly from intact TGF-beta IIb receptor proteins. Peptides can be specifically cleaved by proteolytic enzymes, including, but not limited to plasmin, thrombin, trypsin, chymotrypsin or pepsin. Each of these enzymes is specific for the type of peptide bond it attacks. Trypsin catalyzes the hydrolysis of peptide bonds in which the carbonyl group is from a basic amino acid, usually arginine or lysine. Pepsin and chymotrypsin catalyse the hydrolysis of peptide bonds from aromatic amino acids, such as tryptophan, tyrosine and phenylalanine. Alternate sets of cleaved protein fragments are generated by preventing cleavage at a site which is susceptible to a proteolytic enzyme. For instance, reaction of the ϵ -amino acid groups of lysine with ethyltrifluorothioacetate in mildly basic solution yields blocked amino acid residues whose adjacent peptide bond is no longer susceptible to hydrolysis by trypsin. Proteins can be modified to create peptide linkages that are susceptible to proteolytic enzymes. For instance, alkylation of cysteine residues with β -halo ethylamines yields peptide linkages that are hydrolyzed by trypsin (Lindley, Nature 178: 647 (1956)). In addition, chemical reagents that cleave peptide chains at specific residues can be used. For example, cyanogen bromide cleaves peptides at methionine residues (Gross and Witkip, J. Am. Chem. Soc., 83: 1510 (1961)). Thus, by treating proteins with various combinations of modifiers, proteolytic enzymes and/or chemical reagents, the proteins may be divided into fragments of a desired length with no overlap of the fragments, or divided into overlapping fragments of a desired length.

Fragments can also be chemically synthesized using techniques known in the art such as the Merrifield solid phase Fmoc or t-Boc chemistry. **Merrifield, Recent Progress in Hormone Research 23: 451 (1967)**

5 D. Production of Altered DNA and Peptide Sequences: Random Methods

Amino acid sequence variants of a protein (such as variants of SEQ ID NO.: 8) can be prepared by random mutagenesis of DNA which encodes the protein or a particular portion thereof. Useful methods include PCR mutagenesis and saturation mutagenesis. A library of random amino acid sequence variants can also be generated by the synthesis of a set of
10 degenerate oligonucleotide sequences. Methods of generating amino acid sequence variants of a given protein using altered DNA and peptides are well-known in the art. The following examples of such methods are not intended to limit the scope of the present invention, but merely serve to illustrate representative techniques. Persons having ordinary skill in the art will recognize that other methods are also useful in this regard.

15 PCR Mutagenesis: Briefly, *Taq* polymerase (or another polymerase) is used to introduce random mutations into a cloned fragment of DNA (See Leung et al., Technique 1: 11-15 (1989)). PCR conditions are chosen so that the fidelity of DNA synthesis is reduced by *Taq* DNA polymers using, for instance, a dGTP/dATP ratio of five and adding Mn^{+2} to the PCR reaction.
20 The pool of amplified DNA fragments is inserted into appropriate cloning vectors to provide random mutant libraries.

Saturation Mutagenesis: One method is described generally in Mayers et al., Science, 229: 242 (1989). Briefly, the technique includes generation of mutations by chemical treatment or
25 irradiation of single stranded DNA *in vitro*, and synthesis of a cDNA strand. The mutation frequency is modulated by the severity of the treatment and essentially all possible base substitutions can be obtained.

Degenerate Oligonucleotide Mutagenesis: A library of homologous peptides can be generated
30 from a set of degenerate oligonucleotide sequences. Chemical synthesis of degenerate sequences can be performed in an automatic DNA synthesizer, and the synthetic genes then ligated into an appropriate expression vector. See Itakura et al., **Recombinant DNA**, Proc. 3rd Cleveland Symposium on Macromolecules, pp. 273-289 (A.G. Walton, ed.), Elsevier, Amsterdam, 1981.

E. Production of Altered DNA and Peptide Sequences: Directed Methods

Non-random, or directed, mutagenesis provides specific sequences or mutations in specific portions of a polynucleotide sequence that encodes an isolated polypeptide, to provide
5 variants which include deletions, insertions or substitutions of residues of the known amino acid
sequence of the isolated polypeptide. The mutation sites may be modified individually or in
series, for instance by: (1) substituting first with conserved amino acids and then with more
radical choices depending on the results achieved; (2) deleting the target residue; or (3) inserting
residues of the same or a different class adjacent to the located site, or combinations of options 1-
10 3.

Alanine scanning Mutagenesis: This method locates those residues or regions of a desired
protein that are preferred locations for mutagenesis. See Cunningham and Wells, Science 244:
1081-1085 (1989). In alanine screening, a residue or group of target residues are selected and
15 replaced by alanine or polyalanine. This replacement can affect the interaction of the amino acid
with neighboring amino acids and/or with the surrounding aqueous or membrane environment.
Those having functional sensitivity to the substitutions are then refined by introducing further or
other variants at, or for, the sites of substitution.

Oligonucleotide-Mediated Mutagenesis: One version of this method may be used to prepare
substitution, deletion, and insertion variants of DNA. See Adelman et al., DNA 2:183 (1983).
Briefly, the desired DNA is altered by hybridizing an oligonucleotide primer encoding a DNA
mutation to a DNA template which typically is the single stranded form of a plasmid or phage
containing the unaltered or wild-type DNA sequence template of the desired protein (e.g., the
25 protein). After hybridization, a DNA polymerase is used to make the second and complementary
strand of DNA of the template that will incorporate the oligonucleotide primer, and will code for
the selected alteration in the desired DNA sequence. Generally, oligonucleotides of at least 25
nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are
completely complementary to the template on either side of the mutation. This ensures that the
30 oligonucleotide will hybridize properly to the single-stranded DNA template molecule.

Cassette Mutagenesis: This method (Wells et al., Gene 34: 315 (1985)) requires a plasmid or
other vector that contains the protein subunit DNA to be mutated. The codon(s) in the protein

subunit DNA are identified and there is inserted a unique restriction endonuclease site on each side of the identified mutation site(s), using the above-described oligonucleotide-directed mutagenesis method. The plasmid is then cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures. The two strands are synthesized separately and then hybridized together using standard methods. This double-stranded oligonucleotide is the "cassette" and it has 3' and 5' ends that are compatible with the ends of the linearized plasmid so that it can be directly ligated therein. The plasmid now contains the mutated desired protein subunit DNA sequence.

Combinatorial Mutagenesis: In one version of this method (Ladner et al., WO 88/06630), the amino acid sequences for a group of homologs or other related proteins are aligned, preferably to promote the highest homology possible. All of the amino acids which appear at a given position of the aligned sequences can be selected to create a degenerate set of combinatorial sequences. The variegated library is generated by combinatorial mutagenesis at the nucleic acid level, and is encoded by a variegated gene library. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into the gene sequence such that the degenerate set of potential sequences are expressible as individual peptides, or alternatively, as a set of proteins containing the entire set of degenerate sequences.

IV. Other Variants of Isolated Polypeptides

Derivatives of proteins of the invention also include various structural forms of the primary protein which retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, a TGF-beta IIb receptor fusion protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction. The primary amino acid structure (e.g., of the TGF-beta RIIb moiety) may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like, or by creating amino acid sequence mutants.

Covalent derivatives of the TGF-beta IIb receptor may be prepared by linking particular functional groups to TGF-beta IIb receptor amino acid side chains or at the N- or C-termini. Other derivatives of TGF-beta RIIb:Fc include covalent or aggregative conjugates of TGF-beta RIIb or its fragments with other proteins or polypeptides, such as by synthesis in recombinant

culture as additional N-terminal or C-terminal fusions. For example, the conjugated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast *alpha*-factor leader). TGF-beta IIb receptor proteins can comprise peptides added to facilitate purification or identification of TGF-beta IIb receptor (e.g., poly-His). The amino acid sequence of TGF-beta IIb receptor can also be linked to the peptide Asp-Tyr-Lys-Asp-Asp-Asp-Lys (DYKDDDDK) (Hopp et al., Bio/Technology 6:1204,1988.) The latter sequence is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. This sequence is also specifically cleaved by bovine mucosal enterokinase at the residue immediately following the Asp-Lys pairing. Fusion proteins capped with this peptide may also be resistant to intracellular degradation in *E. coli*.

Polyvalent forms of TGF-beta IIb receptor are useful since they possess multiple TGF-beta IIb receptor binding sites for TGF-beta ligand. For example, a bivalent soluble TGF-beta IIb receptor may consist of two tandem repeats of amino acids 1 to ___ of SEQ ID NO: 2 (or those encoded by nucleic acids numbered ??? to ??? of SEQ. ID.NO.: 1) separated by a linker region, the repeats bound to the immunoglobulin constant domain. Alternate polyvalent forms may also be constructed, for example, by chemically coupling TGF-beta IIb receptor Fc fusions to any clinically acceptable carrier molecule, a polymer selected from the group consisting of Ficoll, polyethylene glycol or dextran using conventional coupling techniques. Alternatively, TGF-beta IIb receptor fusion may be chemically coupled to biotin, and the biotin-TGF-beta IIb receptor Fc conjugate then allowed to bind to avidin, resulting in tetravalent avidin/biotin/TGF-beta IIb receptor molecules. TGF-beta IIb receptor Fc fusions may also be covalently coupled to dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting conjugate precipitated with anti-DNP or anti-TNP-IgM, to form decameric conjugates with a valency of 10 for TGF-beta receptor binding sites.

Functional mutant analogs of the TGF-betaRIIb:Fc fusion having inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A1-Z, where A1 is any amino acid except Pro, and Z is Ser or Thr. In this sequence, Asn provides a side chain amino group for covalent attachment of carbohydrate. Such a site can be eliminated by substituting another amino

acid for Asn or for residue Z, deleting Asn or Z, or inserting a non-Z amino acid between A1 and Z, or an amino acid other than Asn between Asn and A1. TGF-beta IIb receptor derivatives may also be obtained by mutations of TGF-beta IIb receptor or its subunits. Bioequivalent analogs of TGF-beta IIb receptor proteins may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity.

Other analogs include TGF-beta RIIB:Fc protein or its biologically active fragments whose sequences differ from SEQ ID NO: 2 by one or more conservative amino acid substitutions or by one or more non conservative amino acid substitutions, or by deletions or insertions which do not abolish the isolated protein's biological activity. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics such as substitutions within the following groups: valine, alanine and glycine; leucine and isoleucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. The non-polar hydrophobic amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Other conservative substitutions can be readily known by workers of ordinary skill. For example, for the amino acid alanine, a conservative substitution can be taken from any one of D-alanine, glycine, beta-alanine, L-cysteine and D-cysteine. For lysine, a replacement can be any one of D-lysine, arginine, D-arginine, homo-arginine, methionine, D-methionine, ornithine, or D-ornithine.

Generally, substitutions that may be expected to induce changes in the functional properties of isolated polypeptides are those in which: (i) a polar residue, e.g., serine or threonine, is substituted for (or by) a hydrophobic residue, e.g., leucine, isoleucine, phenylalanine, or alanine; (ii) a cysteine residue is substituted for (or by) any other residue; (iii) a residue having an electropositive side chain, e.g., lysine, arginine or histidine, is substituted for (or by) a residue having an electronegative side chain, e.g., glutamic acid or aspartic acid; or (iv) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having such a side chain, e.g., glycine.

Included in the invention are isolated molecules that are: allelic variants, natural mutants, induced mutants, proteins encoded by DNA that hybridize under high or low stringency

conditions to a nucleic acid which encodes a polypeptide such as SEQ. ID. NO. 1. All modifications described herein are expected to retain the biological function of the original splice variant protein.

V. Utilities

This invention provides for administering to an individual with a medical condition a sufficient amount of TGF-beta receptor fusion protein, such as TGF-beta RIIb:Fc, to reduce TGF-beta activity in the individual. This invention further provides for delivering TGF-beta RIIb:Fc to a site where TGF- beta is in excess, such as in disease states characterized by fibroproliferation and immunosuppression such as is associated with infectious disease.

While not wishing to be bound by any particular theory, it appears that the TGF- beta RIIbFc regulates TGF- beta activity by competing for TGF- beta with cell-surface receptors. It is further believed that it inactivates TGF- beta by removing it from the free pool of TGF- beta available to interact with cell surface receptors. Depending on the pharmacologic properties of clearance, the TGF beta -RIIb:Fc/TGF- beta complex is removed from the site of TGF- beta. This complexing with excess TGF- beta reduces the amount of free TGF- beta. With less TGF- beta available to complex with cell receptors, TGF- beta induced fibroproliferation slows down, resulting in stasis of the disease state.

The administration of proteins of the invention fragments of the present invention may be used in fibroproliferative disorders. As mentioned above, animal models of glomerulonephritis have shown good results with anti-TGF- beta antibodies blocking excess TGF- beta . These antibodies will be difficult to deliver because they have a high molecular weight and they may result in severe allergic reactions when they are derived from other species. Thus, it would be preferable to administer a lower molecular weight, native protein or close analog, such as TGF-beta RIIbFc, in glomerulonephritis. Kidney disorders associated with TGF- beta excess include, but are not limited to, mesangial proliferative glomerulonephritis, crescentic glomerulonephritis, diabetic nephropathy, renal interstitial fibrosis, renal fibrosis in transplant patients receiving cyclosporin, and HIV-associated nephropathy. These conditions are associated with overproduction of fibrous tissue formation which administration of TGF-beta RIIbFc should suppress.

As TGF-beta RIIb by itself is not known to have an effect aside from capturing TGF-beta , TGF-beta RIIb:Fc may safely be administered during or at the end of retinal reattachment surgery, which is the most common cause of proliferative vitreoretinopathy (PVR) (Connor et al.

(1989) J. Clin. Invest. 83:1661-1666). Another important fibroproliferative condition is rheumatoid arthritis (RA), which is also associated with excess TGF- beta production. Data indicate that blocking TGF- beta at any time in the development or chronic stages of RA may help stop the progressive deterioration of the joint and bone. Hence, fusions of the present invention may be administered to patients with early joint pain and to patients with prolonged joint pain and deteriorated joints. The current theory is that joint deterioration in RA is due to an overproduction of TGF- beta . Excess TGF- beta has been measured in joints after test animals are injected with streptococcal cell walls, whose presence is believed to cause rheumatoid arthritis (RA). Because anti-TGF- beta antibody blocks arthritic changes in this model, it is believed that fusions of the invention may also have a positive effect. Other conditions associated with excess TGF- beta levels include idiopathic pulmonary fibrosis and myelofibrosis. To complex with excess TGF- beta and to slow the development of excess fibrous tissue, proteins of the invention are intended for administration in these conditions.

The proteins of the present invention may be used to treat collagen vascular disorders that are associated with overproduction of TGF- beta. It is currently believed that there is an overproduction of TGF- beta in collagen vascular disorders, such as progressive systemic sclerosis (PSS), polymyositis, scleroderma, dermatomyositis, eosinophilic fascitis, and morphea. Collagen vascular disorders may also be associated with the occurrence of Raynaud's syndrome. Among other effects, TGF- beta overproduction may also be involved in interstitial pulmonary fibrosis, an end-stage lung disorder which is associated with autoimmune disorders such as systemic lupus erythematosus (SLE) and scleroderma (Deguchi, (1992) Ann. Rheum. Dis. 51:362-65); or it may be caused by chemical contact, allergies to dust and hay fever. A therapeutically effective amount of the proteins of this invention may be administered to neutralize the biologic activity of TGF- beta , which in turn would prevent unwanted fibrosis.

Proteins of the present invention also may be used in preventing overproduction of scarring in patients who are known to form keloids or hypertrophic scars. For example TGF-beta RIIb:Fc may be administered to prevent scarring or overproduction of scarring during healing of various types of wounds including surgical incisions and traumatic lacerations. The TGF-beta RIIbFc fusion may be applied to skin wounds before they are closed to help in healing without scar formation. Proteins of the invention may be placed in surgical abdominal wounds to help prevent adhesion formation which occurs all too commonly after that type of surgery. The present invention provides for the local administration of sufficient fusion to complex with local TGF- beta overproduction and prevent overproduction of healing processes.

TGF- beta excess also has been reported in nasal polyposis, a condition characterized by multiple polyps (Ohno et al. (1992) J. Clin. Invest. 89: 1662-68). Fusion proteins can help lower TGF- beta levels and slow the hyperproliferation that results in polyps. For example, TGF-beta RIIbFc can be administered after polyp surgery to prevent overproduction of scarring and recurrence of polyps and can also be administered to inhibit polyp formation in the intestine.

Proteins of the invention may also be administered following coronary angioplasty, preferably placed along the inside of the affected arteries. According to Karas et al., ((1991) Clin. Cardiol. 14:791-801) restenosis or scarring and reclosing of arteries following coronary angioplasty is seen in approximately one-third of patients operated on. Because the fibrous network which ultimately develops into a scar normally accumulates rapidly, early administration of, for instance, TGF-beta RIIb:Fc would reduce excess TGF- beta in this area and slow excessive proliferation of connective tissue and restenosis.

TGF- beta excess has also been observed in cardiac fibrosis after infarction and in hypertensive vasculopathy. To aid in proper healing without excess scar or fibrous tissue formation, proteins of the invention can be administered in these conditions. TGF- beta excess also has been observed in the tissues of patients receiving radiation therapy. Such tissue is characterized by excess connective tissue development, epithelial thinning and blood vessel occlusion associated with overgrowth of endothelial cells. Administration of an TGF-beta RIIbFc will complex with the excess TGF- beta and will contribute to healing without excessive fibrosis.

VI. Formulation, Administration and Dosage

The formulation, method of administration and dosage of proteins of the invention will depend upon the disorder to be treated, and the medical history of the patient. These factors are readily determinable in the course of therapy. Suitable patients with conditions caused by an excess of TGF- beta can be identified by laboratory tests, medical history and physical findings. TGF- beta excess can be determined directly by immunoassay of the patient's serum or of the affected tissue. Excess TGF- beta can also be determined by bioassays such as the cell proliferation assay described in Kekow et al., (1990) Proc. Natl. Acad. Sci. U.S.A. 87: 8321-25. Excess TGF- beta also can be determined indirectly by measuring the level of TGF- beta mRNA (for example, in the polymerase chain reaction of Kekow et al.).

The medical history may reveal facts which support a diagnosis of fibroproliferative disorder, collagen vascular disorder, immunosuppression, or of potential for problematic wound healing, as in peritoneal adhesions following surgery, or restenosis of blood vessels after coronary

angioplasty. Conditions which are identified as being associated with high levels of TGF- beta and/or proliferation of fibrous tissue are considered to cause TGF- beta excess. Patients may have a wide spectrum of physical findings which are indicative of such disorders. Skin biopsies have been used to test TGF- beta in patients with systemic sclerosis. Swollen, hot joints are seen in arthritis. In accordance with the method of the present invention, the formulation comprises proteins of the invention in an administrable form. The method of the present invention provides for formulating proteins of the invention in modes which are readily apparent to those skilled in the art. Preferably, the proteins of the invention are dissolved in physiologically compatible carriers.

Physiologically compatible carriers for proteins of the invention include intravenous solutions, such as normal saline, serum albumin, 5% dextrose, plasma preparations, other protein-containing solutions and TPN solutions. The preferred carrier for parenteral administration is a sterile, isotonic aqueous solution, such as saline or 5% dextrose. Even more preferred is normal saline with human serum albumin. For use in enhancing the immune response to vaccines, proteins of the invention may be mixed with the vaccine formulation.

Depending on the mode of administration, the proteins of the invention may be in the form of liquid or semi-solid dosage preparations, such as for example, liquids, suspensions or the like. Alternatively, a solution may be placed into an implant, such as an osmotic pump, for the slow release over an extended period of time. Alternatively, proteins of the invention may be provided in sustained release carrier formulations such as semi-permeable polymer carriers in the form of suppositories or microcapsules. See, for instance, U.S. Pat. No. 3,773,919 for microcapsular sustained release matrices including polylactides; Sidmon et al., Biopolymers 22 (1), 547-556 (1983) for copolymers of L-glutamic acid and gamma -ethyl-L-glutamate; Langer et al., J. Biomed. Res. 15, 167-277 (1981) for poly(2-hydroxyethylmethacrylate) or the like.

The mode of administration delivers proteins of the invention to the individual in a safe, physiologically effective manner. proteins of the invention may be given by intraocular, intranasal, subcutaneous, intravenous, intramuscular, intradermal, intraperitoneal, intraarticular, enteral or other conventional routes of administration. In a preferred embodiment, the proteins of the invention of the invention is administered locally to the affected tissue sites by bolus injection or perfusion. For example, for PVR, the preferred mode of administration is a single intraocular injection. Local administration is also preferred in peritoneal wounds to avoid adhesion formation and in other wounds to encourage healing with no keloids or visible scars. For nasal polyposis, nasal drops are preferred. Local and systemic administration are equally preferred in lung

fibrosis (parenteral injection or nasal spray or drops). Rheumatoid arthritis (RA) can be treated by intraarticular or systemic administration.

Systemic administration is the preferred mode of administration in glomerulonephritis and in widespread skin disorders (such as progressive systemic sclerosis, diffuse fascitis, and generalized morphea). Systemic administration also is preferred when proteins of the invention is used to enhance vaccine response. proteins of the invention can be administered with the vaccine by subcutaneous, intramuscular or intradermal injection.

The dose of proteins of the invention to be administered can be readily determined by those skilled in the art, based on the usual patient symptoms discussed above. The dosage of proteins of the invention to be given in a bolus injection is preferred to be between 20 ng and 300 mg. The bolus injection may be repeated over several days, or the proteins of the invention can be continuously infused. If given as an intravenous infusion, the amount of proteins of the invention to be infused over a 24-hour period is about 1 mg to about 100 mg.

The amount of proteins of the invention to administer may also be determined by maintaining the local tissue concentration of TGF- beta at a subnormal level, or about 1-1,000 mu g/ml..

Preferably, proteins of the invention is applied topically, injected at the site of the problem or injected intravenously. Most preferably, proteins of the invention is administered by bolus injection at the site where TGF- beta is to be controlled. By intravenous injection, proteins of the invention should be administered at a rate to maintain a circulating serum concentration sufficient to reduce the TGF- beta excess. Preferably, the patient is started with a relatively low dose of proteins of the invention. The low dose preferably should be continued until the patient's acute phase is ameliorated or adequately improved, as indicated appropriate physical findings and laboratory results. Such improvement may be evident in two to three weeks. In the absence of significant improvement, the dose of proteins of the invention should be increased.

Gene therapy methods of administering the molecules of the invention are also encompassed within the scope of the invention. The term "transformation" or "transform" refers to any genetic modification of cells and includes both "transfection" and "transduction". As used herein, "transfection of cells" refers to the acquisition by a cell of new genetic material by incorporation of added DNA. Thus, transfection refers to the insertion of nucleic acid (e.g., DNA) into a cell using physical or chemical methods. Several transfection techniques are known to those of ordinary skill. In contrast, "transduction of cells" refers to the process of transferring nucleic acid into a cell using a DNA or RNA virus. One or more isolated polynucleotide sequences

encoding one or more TGF-beta RIIbFc proteins contained within the virus may be incorporated into the chromosome of the transduced cell. Alternatively, a cell is transduced with a virus but the cell will not have the isolated polynucleotide incorporated into its chromosomes but will be capable of expressing interferon extrachromosomally within the cell. According to one embodiment, the cells are transformed (i.e., genetically modified) ex vivo. The cells are isolated from a mammal (preferably a human) and transformed (i.e., transduced or transfected in vitro) with a vector containing an isolated polynucleotide such as a recombinant TGF-beta RIIbFc nucleotide operatively linked to one or more expression control sequences for expressing a recombinant secreted protein. The cells are then administered to a mammalian recipient for delivery of the protein in situ. Preferably, the mammalian recipient is a human and the cells to be modified are autologous cells, i.e., the cells are isolated from the mammalian recipient. The isolation and culture of cells in vitro has been reported. According to another embodiment, the cells are transformed or otherwise genetically modified in vivo. The cells from the mammalian recipient (preferably a human), are transformed (i.e., transduced or transfected) in vivo with a vector containing the similar isolated polynucleotide and the protein is delivered in situ.

The isolated polynucleotides encoding the fusion protein are introduced into the cell ex vivo or in vivo by genetic transfer methods, such as transfection or transduction, to provide a genetically modified cell. Various expression vectors (i.e., vehicles for facilitating delivery of the isolated polynucleotide into a target cell) are known to one of ordinary skill in the art. Typically, the introduced material includes an isolated polynucleotide of the invention together with a promoter to control transcription. If delivery of the TGF-beta RIIbFc polynucleotide is to specific tissues, it may be desirable to target the expression of this gene. For instance, there are many promoters described in the literature which are only expressed in certain tissues. Thus, by selecting the appropriate promoter (constitutive versus inducible; strong versus weak), it is possible to control both the existence and level of expression of a fusion protein in the genetically modified cell. If the gene encoding the fusion protein is under the control of an inducible promoter, delivery of the protein in situ is triggered by exposing the genetically modified cell in situ to conditions permitting transcription of the protein, e.g., by injection of specific inducers of the inducible promoters which control transcription of the agent. Recently, very sophisticated systems have been developed which allow precise regulation of gene expression by exogenously administered small molecules. These include, the FK506/Rapamycin system (Rivera et al., *Nature Medicine* 2(9): 1028-1032, 1996); the tetracycline system (Gossen et al., *Science* 268: 1766-1768, 1995), the

ecdysone system (No et al., Proc. Nat. Acad. Sci., USA 93: 3346-3351, 1996) and the progesterone system (Wang et al., Nature Biotechnology 15: 239-243, 1997).

Accordingly, the amount of fusion protein that is delivered in situ is regulated by controlling such factors as: (1) the nature of the promoter used to direct transcription of the inserted polynucleotide (i.e., whether the promoter is constitutive or inducible, strong or weak or tissue specific); (2) the number of copies of the exogenous polynucleotide that are inserted into the cell; (3) the number of transduced/transfected cells that are administered (e.g., implanted) to the patient; (4) the size of an implant (e.g., graft or encapsulated expression system) in ex vivo methods; (5) the number of implants in ex vivo methods; (6) the number of cells transduced/transfected by in vivo administration; (7) the length of time the transduced/transfected cells or implants are left in place in both ex vivo and in vivo methods; and (8) the production rate of the fusion protein by the genetically modified cell.

Expression vectors compatible with mammalian host cells for use in gene therapy include, for example, plasmids; avian, murine and human retroviral vectors; adenovirus vectors; herpes viral vectors; parvoviruses; and non-replicative pox viruses. In particular, replication-defective recombinant viruses can be generated in packaging cell lines that produce only replication-defective viruses. See **Current Protocols in Molecular Biology**: Sections 9.10-9.14 (Ausubel et al., eds.), Greene Publishing Associates, 1989. Specific viral vectors for use in gene transfer systems are now well established. See for example: Madzak et al., J. Gen. Virol., 73: 1533-36 (1992) (papovavirus SV40); Berkner et al., Curr. Top. Microbiol. Immunol., 158: 39-61 (1992) (adenovirus); Moss et al., Curr. Top. Microbiol. Immunol., 158: 25-38 (1992) (vaccinia virus); Muzyczka, Curr. Top. Microbiol. Immunol., 158: 97-123 (1992) (adeno-associated virus); Margulskes, Curr. Top. Microbiol. Immunol., 158: 67-93 (1992) (herpes simplex virus (HSV) and Epstein-Barr virus (EBV)); Miller, Curr. Top. Microbiol. Immunol., 158: 1-24 (1992) (retrovirus); Brandyopadhyay et al., Mol. Cell. Biol., 4: 749-754 (1984) (retrovirus); Miller et al., Nature, 357: 455-450 (1992) (retrovirus); Anderson, Science, 256: 808-813 (1992) (retrovirus). Preferred vectors are DNA viruses that include adenoviruses (preferably Ad-2 or Ad-5 based vectors), herpes viruses (preferably herpes simplex virus based vectors), and parvoviruses (preferably "defective" or non-autonomous parvovirus based vectors, more preferably adeno-associated virus based vectors, most preferably AAV-2 based vectors). See, e.g., Ali et al., Gene Therapy 1: 367-384, 1994; U.S. Patent 4,797,368 and 5,399,346 and discussion below.

Adeno-associated viruses (AAV) have also been employed as vectors for somatic gene therapy. AAV is a small, single-stranded (ss) DNA virus with a simple genomic organization (4.7

kb) that makes it an ideal substrate for genetic engineering. Two open reading frames encode a series of *rep* and *cap* polypeptides. *Rep* polypeptides (*rep78*, *rep68*, *rep 62* and *rep 40*) are involved in replication, rescue and integration of the AAV genome. The *cap* proteins (VP1, VP2 and VP3) form the virion capsid. Flanking the *rep* and *cap* open reading frames at the 5' and 3' ends are 145 bp inverted terminal repeats (ITRs), the first 125 bp of which are capable of forming Y- or T-shaped duplex structures. Of importance for the development of AAV vectors, the entire *rep* and *cap* domains can be excised and replaced with a therapeutic or reporter transgene. See B.J. Carter, in **Handbook of Parvoviruses**, ed., P. Tijsser, CRC Press, pp. 155-168 (1990). It has been shown that the ITRs represent the minimal sequence required for replication, rescue, packaging, and integration of the AAV genome. The AAV life cycle is biphasic, composed of both latent and lytic episodes. During a latent infection, AAV virions enter a cell as an encapsidated ssDNA, and shortly thereafter are delivered to the nucleus where the AAV DNA stably integrates into a host chromosome without the apparent need for host cell division. In the absence of a helper virus, the integrated AAV genome remains latent but capable of being activated and rescued. The lytic phase of the life cycle begins when a cell harboring an AAV provirus is challenged with a secondary infection by a herpesvirus or adenovirus which encodes helper functions that are required by AAV to aid in its excision from host chromatin (B.J. Carter, *supra*). The infecting parental single-stranded (ss) DNA is expanded to duplex replicating form (RF) DNAs in a *rep* dependent manner. The rescued AAV genomes are packaged into preformed protein capsids (icosahedral symmetry approximately 20 nm in diameter) and released as infectious virions that have packaged either + or - ssDNA genomes following cell lysis. AAV have significant potential in gene therapy. The viral particles are very stable and recombinant AAVs (rAAV) have "drug-like" characteristics in that rAAV can be purified by pelleting or by CsCl gradient banding. They are heat stable and can be lyophilized to a powder and rehydrated to full activity. Their DNA stably integrates into host chromosomes so expression is long-term. Their host range is broad and AAV causes no known disease so that the recombinant vectors are non-toxic. Recently, recombinant baculovirus, primarily derived from the baculovirus *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV), has been shown to be capable of transducing mammalian cells in vitro. (See Hofmann, C., Sandig, V., Jennings, G., Rudolph, M., Schlag, P., and Strauss, M. (1995), "Efficient gene transfer into human hepatocytes by baculovirus vectors", Proc. Natl. Acad. Sci. USA 92, 10099-10103; Boyce, F.M. and Bucher, N.L.R. (1996) "Baculovirus-mediated gene transfer into mammalian cells", Proc. Natl. Acad. Sci. USA 93, 2348-2352). Recombinant baculovirus has several potential advantages for gene therapy. These include a very large DNA insert capacity, a

lack of a pre-existing immune response in humans, lack of replication in mammals, lack of toxicity in mammals, lack of expression of viral genes in mammalian cells due to the insect- specificity of the baculovirus transcriptional promoters, and, potentially, a lack of a cytotoxic T lymphocyte response directed against these viral proteins

5 The invention will be illustrated by the following, non-limiting examples.

Example 1: Construction and Expression of Soluble Human Variant TGF β RIIb:Fc Fusion Protein

We generated the extracellular domain of the TGF β RIIb by PCR from mouse lung cDNA. We identified two PCR fragments of approximately the expected length so we cloned and
10 sequenced both. One had the expected sequence. The other had an additional 25 amino acids. We queried the public protein databases with the sequence, and the search resulted in match with the mouse splice variant (Suzuki et al, 1994), and a match with the human (Nikawa et al, 1994) variant.

Specifically, the recombinant human TGF β RIIb:Fc fusion gene, consisting of the splice
15 variant of the extracellular domain of the human type IIb receptor (the domain coding region between nucleotides 832 and nucleotides 2070 of SEQ ID 1) fused to the Fc portion of human IgG1 is constructed as follows:

Fragment 1) a 552 bp fragment , containing a splice variant of the extracellular domain of the human TGF β type II receptor, is amplified from appropriate cDNA (e.g. a cDNA library
20 made from human glioblastoma cells [Nikawa, 1994, Gene 149: 367-372]) by conventional PCR using oligonucleotide primers (SEQ ID NO 3 and SEQ ID NO 4). These oligonucleotides confer flanking Not1 and Sal1 restriction enzyme sites on the resulting PCR product, which is subsequently digested with these two restriction enzymes.

Fragment 2) pSAB132 (Miller et al., *J. Exp. Med.* 178:211 [1993]) is digested with the
25 restriction enzyme Not1, and the terminal phosphate groups are removed by digestion with calf alkaline intestinal phosphatase.

Fragment 3) pSAB144 is constructed. The human IgG1 heavy (H) chain constant region containing the hinge region, the C_H2 and the C_H3 sequences, are isolated with H chain-specific primers by PCR amplification of cDNA from the RNA of COS-7 cells that had been transfected
30 with plasmids containing 3 kilobase pair genomic inserts (Miller et al., *J. Exp. Med.* 178:211 [1993]). Heavy chain specific oligonucleotides primers are designed so that the resulting C_H2 and C_H3 IgG1 PCR amplified sequences were flanked by a 5' Sal1 and a 3' Not1 restriction enzyme recognition site. pSAB144 is digested with the restriction enzymes Not1 and Sal1 to release a 700 base pair fragment containing the Fc portion of human IgG1.

Fragments 1,2, and 3 are ligated together, and transformed into transformation competent bacteria. Plasmids are recovered from the transformants and analyzed by restriction enzyme digestions and gel electrophoresis for the correct orientation of assembled fragments. This construct comprises the recombinant human TGF β RIIb:Fc fusion gene, and the entire coding sequence is confirmed by DNA sequencing (SEQ ID NO 1).

The recombinant human TGF β RIIb:Fc fusion gene is transfected into Chinese hamster ovary (CHO) cells by electroporation at 280 volts. After the initial transfection, cells are selected for the expression dihydrofolate reductase (DHFR) by subculturing in selective medium lacking glycine, hypoxanthine, and thymidine. The resulting colonies are then transferred to 24 well plates and analyzed for human TGF β RIIb:Fc fusion gene expression. The highest expressing cultures are subjected to amplification by exposure to increasing amounts of methotrexate. Cells able to grow at 250 nM methotrexate are cloned by limiting dilution in 96 well plates. The highest expressing clones are transferred to suspension culture and the clone expressing the highest levels of TGF β RIIb:Fc is selected for production of TGF β RIIb:Fc.

Example 2: The Variant TGF β R:Fc Fusion Protein binds TGF β in vitro.

The recombinant variant TGF β RIIb:Fc fusion gene of expression vector SEQ ID NO.: 1 is transfected into COS cells by electroporation at 280 volts. Transfected cells are grown in DMEM, supplemented with 10% fetal bovine serum. After 5 days, the cells are removed from the culture by centrifugation and the supernatant is assessed for the expression of recombinant TGF β RIIb:Fc by SDS/Polyacrylamide gel electrophoresis (SDS/PAGE). TGF β RIIb:Fc present in the cell culture supernatant is tested for the ability to bind TGF β . [125 I]-TGF β (NEN Life Science Products) is incubated for 2.5 hours with conditioned cell culture medium containing TGF β RIIb:Fc, or control IgG, and Protein A-Sepharose which binds to the Fc portion of IgG. The resulting Protein A: Fc complexes are recovered by centrifugation, washed in phosphate buffered saline, and analyzed by SDS/PAGE and autoradiography. TGF β RIIb:Fc, but not control IgG immunoprecipitates [125 I]-TGF β .

As discussed above, there are three members of the TGF-beta family that use the Type II receptor: TGF-beta 1, 2, and 3. The non-variant form of the TGF-beta Type II receptor preferentially binds 1 and 3 (reference). It is surprising to find that the variant form has a different specificity, i.e. Type IId now binds all three TGF-beta's equally. We performed the same test for TGF-beta binding ability as described in the previous paragraph except we used labeled TGF-beta 1, 2 and 3 and found that Type IId now binds all three TGF-beta's equally.

Example 3: Mink Lung Cell Proliferation Assay

The proliferation of mink lung epithelial cells (CCL64) is inhibited by TGF β 1. Thus, we can test the ability of TGF β RIIb:Fc to block the anti-proliferative effect of TGF β 1. CCL64 cells are maintained in MEM supplemented with 100 units/ml penicillin, 100 Tg/ml streptomycin, and 10% fetal bovine serum. To test, serial dilutions of TGF β RIIb:Fc are incubated with 0.1 ng/ml TGF β 1 for 1 hr in assay medium (MEM supplemented with 100 units/ml penicillin, 100 Tg/ml streptomycin, and 10% fetal bovine serum) in a 96-well microtiter tissue culture plate. CCL64 cells are resuspended in assay medium and added to the assay plate at a concentration of 4000 cells per well. The cells are incubated at 37 degrees C for 72 hours and pulsed with [3H] thymidine (70-86 Ci/mmol) for an additional 4 hours. DNA synthesis, which reflects cell proliferation, is determined by measuring incorporation of [3H] thymidine. When cell are allowed to proliferate in media alone, the amount of [3H] thymidine incorporated is measured as counts per minute (CPM). When TGF β 1 is included in the media, proliferation is inhibited, and the amount of [3H] thymidine incorporated measured as counts per minute is diminished.

Example 4: Rat Vessel Restenosis Injury Model

Male Sprague-Dawley rats weighing 400g and about 3-4 months of age (Bantin & Kingman, Edwards, WA) are used throughout. All surgical procedures are carried out under general anesthesia by intraperitoneal injection of xylazine (2.2 mg/kg, AnaSed TM, Lloyd Laboratories, Shenandoah, IA) and ketamine (540 mg/kg, Ketaset TM, Aveco Co., Fort Dodge, IA). The left common carotid artery is denuded with a 2F balloon catheter by introducing the catheter through the external carotid artery. The distal left common carotid and external carotid arteries are exposed through a midline wound in the neck. The catheter is passed three times with the balloon distended sufficiently with saline to generate slight resistance; this method produces distension of the carotid itself. The external carotid is ligated after removal of the catheter and the wound closed.

Experimental treatments include a series of 1 ml intravenous injection of TGF-beta Type IIB receptor:Fc (SEQ ID NO: 2) in PBS given every other day (post-operation) at 5 mg/kg. After 14 days post balloon catheter denudation, all rats are anesthetized and the carotid arteries fixed by perfusion at 120 mm Hg pressure with 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer, pH 7.4 via a large cannula placed retrograde in the abdominal aorta. Ten minutes before fixation, these animals are given an intravenous injection of Evans blue (0.3 ml in

5% saline solution). After 5 minutes of perfusion, the entire left and right common carotid arteries are retrieved, including the aortic arch. The vessels are further fixed by immersion in the same fixative as was used for perfusion.

Arterial segments are assayed for the presence or absence of endothelium by obtaining three segments from the denuded, blue-stained left carotid artery and embedding them in paraffin for cross sectioning using a “Micron” microtome. For measuring intimal areas, photomicrographs are obtained from 3-4 sections from each animal. The photomicrographs are digitized and analyzed with image analysis software (NIH Image 1.55 for MacIntosh). Intimal areas are measured by determining the area between lumen and internal elastic lamina. Medial areas are determined by measuring the area between internal and external elastic lamina. Intimal/medial area ratios are calculated from the measurements.

Example 5: Lung Fibrosis Model

An excess accumulation of collagen in the lungs is a hallmark of lung fibrosis. The amount of collagen in tissue depends on the rate of collagen synthesis and collagen degradation and in cases of fibrosis, the rate of collagen synthesis predominates the rate of collagen degradation.

Chronic respiratory disease free Golden Syrian hamster weighing 120-130g are purchased from Charles River (Boston, MA) and housed in plastic cages in groups of 4 in facilities approved by the American Association for Accreditation of Laboratory Animal Care. The animals are allowed to acclimate for one week to laboratory conditions prior to starting the experiments. They have access to Rodent Laboratory Chow 5001 (Purina Mills, Inc., St. Louis, MO) and water *ad libitum* and housed in a room which gets the filtered air and has 12hr/12hr light/dark cycle. Hamsters are assigned into the following groups for the biochemical and histopathological studies.

| Treatment Group | Biochemical Study (n) | Histopathology Study (n) |
|--|-----------------------|--------------------------|
| Saline IT + Saline IP | 10 | 6 |
| Bleomycin IT + saline IP | 10 | 6 |
| Bleomycin IT + TGF-beta Type IIb receptor:Fc | 10 | 6 |
| Saline IT + TGF-beta receptor | 10 | 6 |

Type IIb: Fc

Bleomycin sulfate is dissolved in pyrogen free sterile isotonic saline just before intratracheal (IT) instillation. Under pentobarbital anesthesia (25-35mg/kg ip) hamsters in appropriate group receive either bleomycin (5.5 units/kg/4ml) or an equivalent volume (4ml/kg) of pyrogen free isotonic saline through transoral route. The TGF- β Type IIb receptor:Fc is administered by intraperitoneal (IP) or intratracheal route at a therapeutic dose to hamster in appropriate groups twice a week for 21-28 days post installation. Thereafter, the animals in each group are killed by an overdose of sodium pentobarbital (100-125 mg/kg ip) and their lungs processed for biochemical and histopathological studies.

1. Biochemical Study

The lungs of animals used for biochemical studies are perfused *in situ* via the right ventricle with ice-cold isotonic saline to wash out blood from the pulmonary vasculature through an opening in the left ventricle. The lung lobes are quickly dissected free of non-parenchymal tissue, dropped in liquid nitrogen for quick freezing and then stored at -80C. The frozen lungs are later thawed and homogenized in 0.1 M KC, 0.02 M Tris buffer (pH 7.6) with a Polytron homogenizer. Several 1 ml aliquots of the homogenate are stored at -80C until the time various biochemical assays are carried out.

Hydroxyproline content of the lung homogenate as a measure of collagen content is quantitated by the techniques of Woessner, Arch. Biochem. Biophys. 93: 440-447 (1961) and lipid peroxidation by the method as described in Giri, et al., Biochem. Pharmacol. 54: 1205-1216 (1997).

2. Histopathological Study

Hamsters are anesthetized to a level of cardiac cessation. The thorax is opened, the heart tied off at its base and the trachea cannulated. The lungs and heart will be removed *en bloc*, weighed in place in a normal saline bath and the lungs instilled with a 1% glutaraldehyde-paraformaldehyde fixative in 0.12M cacodylate buffer at 400m Osm at 30 cm H₂O pressure. The lungs are fixed via this pressure for about 2 hours and then stored in fixative with the tracheas occluded. Before embedding, the lung is isolated from the heart and all non-pulmonary tissue by blunt dissection and removed. Blocks of tissue are cut from at least two sagittal slabs (2-3mm thick) from the right cranial, right caudal, and left lung lobes of each lung. Each block is cut with

about a 1 cm² face. The blocks are dehydrated in a graded series of ethanol and embedded in paraffin. Sections (5µm thick) are cut from the paraffin blocks and stained with haematoxylin and eosin for histological evaluations.

5 3. Data Analysis and Interpretation

The values for different biochemical determinants in the lungs of control and treated animals are expressed per mg lung protein or DNA. However, it should be noted that the expression of the data based on per mg of lung protein or DNA will tend to introduce artificial lowering of the values in treated animals. This problem is attributed to the infiltration of
10 leukocytes, presence of residual plasma protein in the lungs even after perfusion, and proliferation of type II cells and fibroblasts in a long term study in the lungs of hamster treated with bleomycin. In order to avoid an artificial lowering of the values, the data are expressed on a per lung basis. See Starcher et al., Am. Rev. Resp. Dis., 117:299-305 (1973) and Witschi, Essays Toxicol., 6:125-191 (1975).

15 The data are analyzed in terms of average values with their standard deviations and standard errors of means. Student's t-test, chi-square distributions, correlation coefficient, analysis of variance (ANOVA) and multiple comparison test will be applied to judge the significance of differences between the control and treatment groups using a computer based statistical package (SAS/STAT Guide, 6th Ed. Cary, N.C. pp. 183-260 (1985)).

20 Example 6: Glomerulonephritis Model

The effect of proteins of the invention is assessed in a glomerulonephritis model. Experimental glomerulonephritis is induced in rats with a single injection of anti-glomerular basement membrane nephrotixin serum (NTS), derived in rabbits. The experimental lesion is
25 acute mesangial proliferative glomerulonephritis and is characterized by expansion of the mesangial matrix and hypercellularity. The injured cells also express more TGF- beta 1 mRNA and TGF- beta 1, which in turn stimulates the synthesis of two proteoglycans, biglycan and decorin. Of particular interest, the nephritis reproducibly progresses through glomerular and tubulointerstitial scarring, to end stage renal disease.

30 First, glomerulonephritis is induced in rats by an intravenous injection of NTS serum. Next, for six days, two groups of rats are treated with daily intravenous injections of saline (the negative control group) or proteins of the invention. On the tenth day, the animals are sacrificed and slides are made of the kidneys, which are stained with periodic acid-Schiff solution to

emphasize the pathological changes. The negative control kidneys have full-blown glomerulonephritis with reddish-pink amorphous fibrous material filling most of the glomerulus. The positive control kidneys have a staining pattern which is similar to a normal glomerulus. The kidney which is treated with proteins of the invention also has a normal appearance, indicating that the proteins of the invention blocks the response due to the secretion of overproduction of TGF- beta .

The extent of glomerular injury is quantitated by performing glomerular cell counts from 30 randomly selected glomeruli from normal animals and nephritic animals in each group. By day 10, there are more cells in glomeruli from NTS-treated rats. Kidney function is also assessed by measuring levels of albumin in urine and by quantitating creatinine clearance.

Another measure of the effect of proteins of the invention on the disease process is to quantitate the amount of extracellular matrix accumulation in the glomeruli. The degree of glomerular matrix expansion is determined as the percentage of each glomerulus occupied by the mesangial matrix according to the method of Raij et al. (1984) *Kidney Int.* 26: 137-43. The TGF-beta Type IIb:Fc-treated kidneys are expected to have similar percentages of mesangial matrix to that in normal kidney, and significantly less mesangial matrix than in the negative control kidneys.

Example 7: Arthritis Model

Arthritis is induced in pathogen-free female LEW rats (Harlan Sprague Dawley, Indianapolis, Ind.) weighing about 100 grams. Each receives a dose of cell wall fragments from Group A streptococci (SCW) (30 mu g rhamnose/gm bodyweight), injected intraperitoneally (ip) according to the technique described in Brandes et al. (1991) *J. Clin. Invest.* 87:1108. SCW-injected and control LEW rats are given an intraarticular (IA) injection in one of the hind ankles of one of the following:

1. proteins of the invention in 25 mu l PBS,
2. PBS only, or
3. an IgG control

Joints are clinically monitored by determining the amount of joint erythema, swelling and distortion on a scale of 0 (normal) to 4 (severe inflammation). Radiographs are taken and are evaluated for soft tissue swelling, joint space narrowing, bone erosions and deformity. Tissue specimens are obtained and prepared for histopathologic analysis as described in Brandes et al., *ibid.* Total RNA is isolated from excised synovial tissues according to the method of Allen et al. (1990) *J. Exp. Med.* 171:231.

Injection of SCW produces an acute inflammatory response which is clinically detectable within hours and maximal in 3-5 days. When variant TGF-beta Type IIb receptor:Fc is injected directly into a joint before ip administration of the SCW, inflammation at 24 hours is significantly below that observed in joints with the irrelevant antibody. At the peak of the acute response, inflammation of such treated joints remains far below that of joints with the irrelevant antibody. Even if joints are injected with variant TGF-beta Type IIb receptor:Fc when inflammation is well developed, the fusion still has a significant anti-inflammatory effect, when compared to irrelevant antibody. Because proteins of the invention also bind TGF-beta, they have a similarly beneficial effect when given early or late in the inflammatory process. Other well established experimental models of RA include collagen and adjuvant-induced arthritis. See, for example Janusz MJ et al., *Inflamm Res* 46:503-508 (1997); Myers et al., *Life Sci* 61:1861-1878 (1997); and Kock et al., *Clin Immunol Immunopathol* 86: 199-208 (1998).

EQUIVALENTS

This invention has been detailed both by example and by direct description. It should be apparent that one having ordinary skill in this art would be able to surmise equivalents to the invention as described in the claims which follow but which would be within the spirit of the description above. Those equivalents are to be included within the scope of this invention.